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EXAMINER	
KOROMA, BARBA M	
ART UNIT	PAPER NUMBER
1638	

DATE MAILED: 02/10/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/808,979	CHENG ET AL.	
	Examiner	Art Unit	
	Barba M. Koroma	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 November 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *6 and 13-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *6 and 13-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/14/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Sequence compliance correspondence sent out in last action is in error and is hereby VACATED.

2. Applicant's election with traverse of Group III, claims 6, 14 and 16-18, filed in the paper filed on November 19, 2004, is acknowledged. Upon further consideration of Applicant's arguments in support of a request for inclusion of claim 13 (amended) and claim 15, with the currently prosecuted claims of elected Group III outlined above, it was determined that it would not be burdensome for the Office to further examine claims 13 (amended) and 15, as requested. Therefore, the restriction requirement as applied to claims 13 and 15 is hereby withdrawn. Claims 6, 13, 14, 15, and 16-18, have been examined in this Office action. Claims 15, 7-12, and 19-32 have been cancelled.

Specification

3. The disclosure is objected to because of the following informalities: The word "herby" preceding the word "incorporated" on line 5, page 1, of the specification, is incorrect. Replacement of "herby" with --hereby-- is suggested.

Claim Rejections - 35 USC 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claim 17 recites the limitation "the transformed host cell" in line 1. There is insufficient antecedent basis for this limitation in claim 16, which is drawn to a transformed host. It is suggested that claim 16 be amended to read --A transformed host cell--.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 6, 13, 14, 15, 16, 17, 18, a re rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contain subject matter which were not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. There is lack of written description of the claims by the specification regarding an isolated nucleic acid comprising the *crtE, crtX, crtY, crtI, crtB,* and *crtZ* gene cluster encoding all of the polypeptides crtE, crtX, crtI, crtY, crtB, and crtZ.

The claims are broadly drawn to an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes from any source, encoding the crtE, crtX, crtY, crtI, crtB, and

crtZ polypeptides from any source, and of any sequence, used as chimeric genes to transform a host cell under the control of suitable regulatory sequences.

In example 4, page 61, the specification describes the introduction of the *crt* gene cluster comprising the *crt*EXYIB genes from bacterial strain DC260 (described in example 3, page 57) into the bacterial host *Methylomonas* 16a (ATCC PTA 2402), to enable the synthesis of desirable 4-carbon carotenoids, such as β-carotene (lines 9-12, page 61). However, the specification does not describe the introduction of the *crt* gene cluster including the *crtZ* gene from bacterial strain DC260 or any other source, into *Methylomonas* 16a (ATCC PTA 2402), or any host cell.

The Federal Circuit has recently clarified the application of the written description requirement. The court stated that a written description of an invention "requires a precise definition, such as by structure, formula, [or] chemical name, of the claimed subject matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 119 F.3d 1559, 1568; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). The court also concluded that "naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material." Id. Further, the court held that to adequately describe a claimed genus, Patent Owner must describe a representative number of the species of the claimed genus, and that one of skill in the art should be able to "visualize or recognize the identity of the members of the genus." Id.

See MPEP Section 2163, page 156 of Chapter 2100 of the August 2001 version, column 2, bottom paragraph, where it is taught that:

[T]he claimed invention as a whole may not be adequately described where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function. A biomolecule sequence described only by a

functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence.

Given the breadth of the claims and lack of guidance as discussed above, the specification fails to provide an adequate written description of the genus of sequences as broadly claimed. Given the lack of written description of the claimed genus of sequences, including the sequence for the *crtZ* gene, any method of using them, such as for transforming *Methylomonas*, or plant cells and plants therewith, and the resultant products including the claimed transformed bacterial cell, plant cells, and plants containing the genus of sequences, would also be inadequately described. Accordingly, one skilled in the art would not have recognized Applicant to have been in possession of the claimed invention at the time of filing. See the Written Description Requirement guidelines published in Federal Register/ Vol. 66, No. 4/ Friday January 5, 2001/ Notices: pp. 1099-1111.

See also Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ 2d 1016 at 1021, (Fed. Cir. 1991) where it is taught that a gene is not reduced to practice until the inventor can define it by "its physical or chemical properties" (e.g. a DNA sequence).

See also University of California v. Eli Lilly and Co., 43 USPQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism which would encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism.

6. Claims 6, 13, 14, 15, 16, 17, 18, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The specification does not describe an isolated nucleic acid comprising the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes, and all its corresponding polypeptides, having at least 95% identity to SEQ ID No. 18.

The claims are broadly drawn to an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB,* and *crtZ* genes encoding all of the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides; or an isolated nucleic acid having at least 95% identity to SEQ ID No. 18, used to transform a host cell under the control of suitable regulatory sequences.

The specification teaches that a 6999bp contig sequence (SEQ ID No. 18) containing carotenoid synthesis genes from DC260 was assembled (figure 5) (example 3, p57). In example 4, page 61, the specification teaches that a fragment from DC620 containing the *crtEXYIB* genes were amplified by PCR to produce a single 5.8kB product.

However, the specification does not teach a fragment that is at least 95% identical to SEQ ID No. 18. Although, the specification mentions the term "fragment" on line 16, page 61, it is not clear whether said fragment is 95%, 96%, 97%, 98%, or 99.5% of the full length of the nucleotide sequence cloned in DC620. The specification teaches a 6999bp identified as SEQ ID No. 18 (page 57, lines 18-19, see also figure 5). However, it is not clear whether this 6999bp (SEQ ID No. 18) sequence is a full-length nucleotide sequence bearing the entire gene cluster, or a fragment thereof.

Since SEQ ID No. 18 is a 6999bp contig sequence, it means that a sequence that is 95% different will in fact differ from it by 350 nucleotides, anywhere in the 6999bp sequence. The specification does not set forth the sequence of even one such variant sequence. Therefore, the specification does not adequately describe the broadly claimed genus of sequence variants.

The Federal Circuit has recently clarified the application of the written description requirement. The court stated that a written description of an invention "requires a precise definition, such as by structure, formula, [or] chemical name, of the claimed subject matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 119 F.3d 1559, 1568; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). The court also concluded that "naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material." Id. Further, the court held that to adequately describe a claimed genus, Patent Owner must describe a representative number of the species of the claimed genus, and that one of skill in the art should be able to "visualize or recognize the identity of the members of the genus." Id.

See MPEP Section 2163, page 156 of Chapter 2100 of the August 2001 version, column 2, bottom paragraph, where it is taught that

[T]he claimed invention as a whole may not be adequately described where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function. A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the

sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence.

Given the lack of written description of the plethora of nucleotide sequences or claimed genus of sequences that comprises the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes encoding all of the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides; or an isolated nucleic acid having at least 95% identity to SEQ ID No. 18, used to transform a host cell such as a plant cell or plant therewith, under the control of suitable regulatory sequences, the resultant products including the claimed transformed plant cells and plants containing the genus of sequences, would also be inadequately described. Accordingly, one skilled in the art would not have recognized Applicant to have been in possession of the claimed invention at the time of filing. See the Written Description Requirement guidelines published in Federal Register/ Vol. 66, No. 4/ Friday January 5, 2001/ Notices: pp. 1099-1111.

See also Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ 2d 1016 at 1021, (Fed. Cir. 1991) where it is taught that a gene (which includes a promoter) is not reduced to practice until the inventor can define it by "its physical or chemical properties" (e.g. a DNA sequence).

See also University of California v. Eli Lilly and Co., 43 USPQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism which would encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism.

7. Claim 16 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The specification does not describe the transformation of any and all multicellular hosts including those of plants and animals.

The claims are broadly drawn to a transformed host comprising the isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes, encoding all the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides, wherein said host encompasses multicellular organisms including plants and animals.

The specification describes the isolation and identification of a yellow-pigmented bacterium designated as strain DC620 which belongs to the family *Enterobacteriaceae* (example 1, page 54). The *crt* gene cluster comprising the *crt*EXYIB genes from DC620 were introduced into *Methylomonas* 16a bacterial strain (ATCC PTA 2402), to express carotenoids such as beta carotene.

However, the specification does not teach the transformation of animals and plants using SEQ ID No. 18 or a fragment thereof to produce beta-carotenes.

The Federal Circuit has recently clarified the application of the written description requirement. The court stated that a written description of an invention "requires a precise definition, such as by structure, formula, [or] chemical name, of the claimed subject matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 119 F.3d 1559, 1568; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). The court also concluded that "naming a type of material generally known to exist, in the absence of knowledge as to what that

material consists of, is not a description of that material." Id. Further, the court held that to adequately describe a claimed genus, Patent Owner must describe a representative number of the species of the claimed genus, and that one of skill in the art should be able to "visualize or recognize the identity of the members of the genus." Id.

See MPEP Section 2163, page 156 of Chapter 2100 of the August 2001 version, column 2, bottom paragraph, where it is taught that

> [T]he claimed invention as a whole may not be adequately described where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function. A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence.

Given the breadth of the claims encompassing any and all possible hosts (including plants and animals) and lack of guidance as discussed above, the specification fails to provide an adequate written description of the genus of hosts as broadly claimed. Accordingly, one skilled in the art would not have recognized Applicant to have been in possession of the claimed invention at the time of filing. See the Written Description Requirement guidelines published in Federal Register/ Vol. 66, No. 4/ Friday January 5, 2001/ Notices: pp. 1099-1111.

See also Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ 2d 1016 at 1021, (Fed. Cir. 1991) where it is taught that a gene (which includes a promoter) is not reduced to practice until the inventor can define it by "its physical or chemical properties" (e.g. a DNA sequence).

See also University of California v. Eli Lilly and Co., 43 USPQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the

actual cDNA from that organism which would encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism.

8. Claims 6, 13, 14, 15, 16, 17, 18, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The specification does not teach how to make and use an isolated nucleic acid comprising the *crtE, crtX, crtY, crtI, crtB,* and *crtZ* genes encoding all the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides.

The claims are broadly drawn to an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB,* and *crtZ* genes encoding all crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides, used to transform a host cell under the control of suitable regulatory sequences.

In example 4, on page 61, the specification teaches the introduction of the *crt* gene cluster comprising the *crt*EXYIB genes from bacterial strain DC260 (described in example 3, page 57) into *Methylomonas* 16a (ATCC PTA 2402) to enable the synthesis of desirable 4-carbon carotenoids, such as β-carotene (lines 9-12, page 61). Transformants containing plasmid pDCQ329 were selected in kanamycin-containing medium, and transferred into *Methylomonas* 16a by triparental conjugal mating. The *Methylomonas* 16a MWM1000 strain contained a single crossover knockout of the ald/crtN1 genes, which disrupted the synthesis of the native C_{30} carotenoids (lines 10-14, page 62). HPLC analysis of extracts from *Methylomonas* 16a containing pDCQ329 showed almost exclusive production of b-carotene. This confirmed the

synthesis of C_{40} carotenoids in the methanotrophic host using the crtEXYIB gene cluster from DC260 (lines 5-10, page 64). Furthermore, the specification teaches that a 6999bp contig (SEQ ID No. 18) containing carotenoid synthesis genes from DC260 was assembled (figure 5). However, the specification does not indicate that this was the only nucleotide sequence from strain DC620 which was made. In addition, it does not specifically teach the utilization of SEQ ID No. 18 in the transformation of *Methylomonas* 16a (ATCC PTA 2402) or any host cell, for biosynthesizing desirable carotenoids such as β-carotene. Further, the specification remains silent on whether the *crt*EXYIB gene cluster utilized in the transformation process (described in example 4, page 61), is synonymous to SEQ ID No. 18.

The specification does not teach how to introduce the *crt* gene cluster comprising the *crt*EXYIBZ genes from bacterial strain DC260 into *Methylomonas* 16a (ATCC PTA 2402) or any host cell, thereby enabling the synthesis of desirable carotenoids, such as β-carotene. Furthermore, the specification does not teach what enzyme is encoded by *crtZ*, or what effect this enzyme will have on the structure of carotenoid intermediates. Thus, one in the art would not know how to use the invention.

The claimed invention is not supported by an enabling disclosure taking into account the *Wands* factors. *In re Wands,* 858/F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). *In re Wands* lists a number of factors for determining whether or not undue experimentation would be required by one skilled in the art to make and/or use the invention. The factors to be considered are as follows: *Lack of guidance:* The specification does not teach how to make the crtZ protein protein when the *crtZ* nucleotide sequence encoding it is missing in the nucleotide template. *State of the prior art*: The method of generating a protein from a template that does not have the

corresponding nucleotide sequence is not taught in the prior art. *Nature of the invention*: Since gene expression in cells require the presence of the gene in order to produce the corresponding protein (or polypeptide), one skilled in the art would not know how to make and use crtE, crtX, crtY, crtI, crtB, crtZ polypeptides without the corresponding nucleotide template.

U*npredictability of the art*: Walden and Schell (1990. Eur. J. Biochem. 192. 563-576. p566, last paragraph) teach that variation in the levels of gene expression of a gene construct occurs in transformed cells as a result of position effect, a phenomenon which may be due to insertion of DNA into a region of the genome not normally transcribed, or near sequences which control expression in a positive or negative manner. Also, Armstrong, G. (1997. Ann. Rev. Microbiol. 51:629-659) teaches that the *crtZ* gene is found only in a few bacterial species (see page 636, Table 2), wherein the encoded crtZ enzyme is required for the production of particular xanthophyll pigments (see page 641, Figure 4). Thus, it is unlikely that transferring only the *crtEXYIB* genes to a multitude of non-exemplified hosts, not containing their own native *crtZ* gene, would result in expression of the *crtZ* polypeptide and the production of the particularly desired xanthophylls. The unpredictability inherent in recombinant production of carotenoids is further demonstrated by Armstrong (page 640, last sentence), where it is taught that the transfer of a *crt* gene cluster from one bacterial species to another did not result in any production of carotenoids. Moreover, Armstrong teaches that different *crt* operons are differentially regulated (see page 648, top paragraph), and there is uncertainty regarding which coding sequences belong to which operon (Armstrong, p636, bottom paragraph), further confounding the claimed process.

Q*uantity of experimentation necessary*: It would require numerous experimental approaches, using different configurations of *crtEXYIB* genes and polypeptides, in different

animal and plant cell models, and under different conditions, to determine whether *crt*EXYIBZ genes and polypeptides can be generated from a *crt*EXYIB gene cluster. In the absence of this information, one skilled in the art would not know how to make and use the invention as claimed. See Genentech, Inc. v. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997), which teaches that "the specification, not the knowledge of one skilled in the art" must supply the enabling aspects of the invention. Given the breadth of the claims encompassing the transformation of any host cell with a *crt*EXYIB gene cluster, to generate *crt*EXYIBZ genes and polypeptides, the lack of guidance of the specification as discussed above, it would require undue experimentation to make and use the invention as claimed.

9. Claims 6, 13, 14, 15, 16, 17, 18, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The specification does not teach how to make and use an isolated nucleic acid comprising the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes encoding all its corresponding polypeptides, or a nucleotide sequence having at least 95% identity to SEQ ID No. 18.

The claims are broadly drawn to an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes encoding all the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides; or an isolated nucleic acid having at least 95% identity to SEQ ID No. 18 comprising the *crtE, crtX, crtY, crtI,crtB, crtZ,* genes encoding all of the corresponding polypeptides, used to transform a host cell under the control of suitable regulatory sequences.

The specification teaches that a 6999bp contig sequence (SEQ ID No. 18)-containing carotenoid synthesis genes from DC260 was assembled (figure 5) (example 3, p57). In example 4, page 61, the specification indicates teaches that a fragment from DC620 containing the *crtEXYIB* genes were amplified by PCR to produce a single 5.8kB product. Transformants containing plasmid Pdcq329 were selected in kanamycin-containing medium, and transferred into *Methylomonas* 16a by triparental conjugal mating. The *Methylomonas* 16a MWM1000 strain contained a single crossover knockout of the ald/crtN1 genes, which disrupted the synthesis of the native C_{30} carotenoids (lines 10-14, page 62). HPLC analysis of extracts from *Methylomonas* 16a containing pDCQ329 showed almost exclusive production of b-carotene, confirming the synthesis of C_{40} carotenoids (lines 5-10, page 64).

The specification does not teach the transformation of *Methylomonas* 16a (ATCC PTA 2402) with a *crt* gene cluster that is less than 100% or at least 95% identical to the full length sequence, whereby such a sequence retains the capacity to express fully the *crt*EXYIBZ genes encoding all the corresponding polypeptides. Although the specification mentions the term "fragment" on line 16, page 61, it is not clear whether the fragment referred to is 95%, 96%, 97%, 98%, or 99.5% of the full length of the nucleotide sequence cloned in DC620. Furthermore, the specification teaches a 6999bp contig sequence identified as SEQ ID No. 18 (page 57, lines 18-19, see also figure 5). However, it is not clear whether this 6999bp (SEQ ID No. 18) sequence is a full-length nucleotide sequence expressing the entire gene cluster or a fragment thereof. Since SEQ ID No. 18 is a 6999bp sequence, it means that a sequence that is 95% different will differ from it by 350 nucleotides. Furthermore, the specification does not teach which of the 350 nucleotides can be altered without destroying or abolishing the property

to express the full complement of the genes encoded by SEQ ID No. 18. Finally, the specification does not describe the utilization of a nucleotide sequence that is 95% identical to SEQ ID No. 18 to transform a host cell in order to express carotenoid genes.

The claimed invention is not supported by an enabling disclosure taking into account the *Wands* factors. *In re Wands,* 858/F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). *In re Wands* lists a number of factors for determining whether or not undue experimentation would be required by one skilled in the art to make and/or use the invention. The factors to be considered are as follows: *Lack of guidance*: The specification does not teach how to make and use a sequence that is at least 95% identical to SEQ ID No. 18 having the properties of expressing *crt*EXYIBZ genes and polypeptides in a transformed host cell. *State of the prior art*: The prior art does not teach the transformation of a host cell with a nucleotide sequence that is at least 95% of SEQ ID No. 18.

Unpredictability of the art: Walden and Schell (1990. Eur. J. Biochem. 192. 563-576. p566, last paragraph) teach that variation in the levels of gene expression of a gene construct occurs in transformed cells as a result of position effect, a phenomenon which may be due to insertion of DNA into a region of the genome not normally transcribed, or near sequences which control expression in a positive or negative manner. Furthermore, the claims do not specify where in SEQ No. 18, the sequence substitutions occur. They could be spread out over each gene in the operon, or be concentrated in one or two genes, completely inhibiting their expression, and thus changing the character of the carotenoid end product, or abolishing carotenoid production completely. (See earlier citation of Armstrong, G. 1997. Ann. Rev. Microbiol. 51:629-659). In addition, even minor substitutions in each gene of the operon could

severely alter the function of the encoded enzyme. See Brown et al (1998. Science 282:1315-1318, especially abstract, p1315), who teach that as few as four amino acid substitutions completely change the enzymatic activity of the encoded protein.

Quantity of experimentation necessary: It would require undue experimentation by one skilled in the art to test a plethora of cDNAs having at least 95% identity to SEQ ID No. 18, in which 350 bases have been rearranged in order to determine which of the fragments express the *crt*EXYIBZ genes and polypeptides. In the absence of this information, it is unclear how one skilled in the art would make and use the invention. See Genentech, Inc. v. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997), which teaches that "the specification, not the knowledge of one skilled in the art" must supply the enabling aspects of the invention.

Given the breadth of the claims encompassing an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB, and crtZ* genes, encoding all crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides; or an isolated nucleic acid having at least 95% identity to SEQ ID No. 18, to transform a host cell under the control of suitable regulatory sequences, the lack of guidance of the specification as discussed above, it would require undue experimentation to make and use the invention as claimed.

10. Claim 16 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for transforming bacterial hosts including *Methylomonas*, does not reasonably provide enablement for all hosts including plants and animals. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are broadly drawn to an isolated nucleic acid that comprises the *crtE, crtX, crtY, crtI, crtB, crtZ* genes encoding all the crtE, crtX, crtY, crtI, crtB, and crtZ polypeptides, used to transform any multicellular host including plants and animals, under the control of suitable regulatory sequences.

In example 4, page 61, the specification teaches the introduction of the *crt* gene cluster comprising the *crtEXYIB* genes from bacterial strain DC260 (described in example 3, page 57) into *Methylomonas* 16a (ATCC PTA 2402), to enable the synthesis of desirable 4-carbon carotenoids, such as β-carotene (lines 9-12, page 61). Transformants containing plasmid pDCQ329 were selected in kanamycin-containing medium, and transferred into *Methylomonas* 16a by triparental conjugal mating. The *Methylomonas* 16a MWM1000 strain contained a single crossover knockout of the ald/crtN1 genes, which disrupted the synthesis of the native C_{30} carotenoids (lines 10-14, page 62). HPLC analysis of extracts from *Methylomonas* 16a containing pDCQ329 showed almost exclusive production of b-carotene, confirming the synthesis of C_{40} carotenoids in DC260 (lines 5-10, page 64).

The specification does not enable one skilled in the art to introduce the *crt* gene cluster comprising the *crtEXYIBZ* genes from bacterial strain DC260 into any other multicellular host. Thus, the claimed invention is not supported by an enabling disclosure taking into account the *Wands* factors. *In re Wands,* 858/F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). *In re Wands* lists a number of factors for determining whether or not undue experimentation would be required by one skilled in the art to make and/or use the invention. The factors to be considered are as follows: *Lack of guidance*: The specification does not teach methods or regulatory sequences

for the transformation of any other host using SEQ ID No. 18, or isolated sequences having at least 95% identity to SEQ ID No. 18. *State of the prior art*: The prior art does not teach methods or regulatory sequences for transformation of any other host using SEQ ID No. 18, or isolated sequences having at least 95% identity to SEQ ID No. 18.

Unpredictability of the art: Bosch et al (1994. Transgenic Research. 3: p304-310) teach that transgenic expression of genes in heterologous eukaryotic hosts could lead to misfolding and partial breakdown of the protein in the host. (see page 307, paragraph bridging the columns; page 308, column 1, 2nd and 3rd paragraphs; paragraph bridging pages 308 and 309; and page 309, clumn 1, first two full paragraphs). See also Armstrong cited previously (p640), which teaches a lack of carotenoid production when a *crt* gene cluster is transferred into a heterologous host. See also Armstrong, first full paragraphs of pages 647 and 649, where it is taught that induction or repression of carotenoid biosynthesis varies greatly between bacterial species. See also Armstrong, page 644, bottom paragraph, where it is taught that different species even within a single host type, namely bacteria, lack essential *crt* genes like *crtE*. *Quantity of experimentation necessary*: It would require undue experimentation by one skilled in the art to test any and all host cells to determine whether SEQ ID No. 18, or isolated sequences having at least 95% identity to SEQ ID No. 18, would express crtEXYIBZ genes and polypeptides. In the absence of this information, one skilled in the art would not know how to make and use the invention as claimed. See Genentech, Inc. v. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997), which teaches that "the specification, not the knowledge of one skilled in the art" must supply the enabling aspects of the invention. Given the breadth of the claims encompassing the transformation of any and all host cells using an isolated nucleic acid that

comprises *crtE, crtX, crtY, crtI, crtB, crtZ* genes and *crtE, crtX, crtY, crtI, crtB, crtZ* polypeptides, having at least 95% identity to SEQ ID No. 18, the lack of guidance of the specification as discussed above, it would require undue experimentation to make and use the invention as claimed.

11. Claims 6, 13, 14, 15, 16, 17, 18, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Since strain DC260 is essential to the claimed invention, it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public (37 CFR 1.801-1.809). The specification does not teach a repeatable process to obtain pJB1178-29. If the E. coli strain comprising the cloned plasmid was deposited under the terms of the Budapest Treaty, then an affidavit or declaration by the applicant (s), or a statement by the attorney of record over his/her signature and registration number, stating that the plasmid will be irrevocably and without restriction or condition be released to the public upon the issuance of a patent, must be submitted. See 37 CFR 1.801-1.809. Mere reference to a deposit or the biological material itself in any document or publication does not necessarily mean that the deposited biological material is readily available. Even a deposit made under the Budapest Treaty and referenced in a United States or foreign patent document would not necessarily meet the test for known and readily available unless the deposit was made under conditions that are consistent with those specified in these rules, including the provision that requires, with one possible exception (37

CFR 1.808(b)), that all restrictions on the accessibility be irrevocably removed by the applicant upon the granting of the patent. *Ex parte Hildebrand, 15 USPQ2d 1662 (Bd. Pat. App. & Int. 1990).* As discussed, it appears that the deposit was made under the Budapest Treaty. However, the aforementioned statement was not received.

If the deposit was not made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 CFR 1.801-1.809, Applicants may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number showing that:

a) during the pendency of the application, access to the invention will be afforded to the Commissioner upon request; b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent; c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the enforceable life of the patent, whichever is longer; d) the viability of the biological material at the time of the deposit will be tested (see 37 FCR 1.807); and e) the deposit will be replaced if it should ever become inviable.

Conclusion

12. Claims 6, 13, 14, 15, 16, 17, 18, are deemed free of the prior art given the failure of the prior art to teach or fairly suggest SEQ ID No. 18, or isolated sequences having at least 95% identity to SEQ ID No. 18; or a vector or host transformed therewith.

Contact Information

13. Any inquiry concerning this or earlier communications from the Examiner should be directed to Barba M. Koroma, whose telephone number is 571-272-0899. The Examiner can normally be reached from 8:00 A.M to 5:30 P.M. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Amy Nelson, can be reached at 571-272-0804. The fax phone numbers for the organization where this application or proceeding is assigned are 571 273 8300. Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov.

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